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The Quantitative Estimation of Lactose in Milk

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THE QUANTITATIVE ESTIMATION OF LACTOSE IN MILK

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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THE QUANTITATIVE ESTIMATION OF LACTOSE IN MILK.

INTRODUCTION.

Milk sugar was first mentioned by Fabricio Bartoletti at Bologna in the year 1615, in 1700 by Testi and by Vallisneri in 1715. They stated that milk sugar is found in the milk of mammals, and especially in cows milk, in which casein is precipitated by rennet, the filtrate evaporated to a syrup and the sugar crystallised from water in sharp irregular crystals.

The importance, in milk analysis, of determining milk sugar accurately was recognized as early as 1876. Lactose is the first substance to suffer change by decomposition of milk and loss in total solids is thus occasioned. By transformation of lactose into lactic acid, discrepancies arise in the analyses of stale milks.

In the Analyst for 1876, we find this statement; " As there is no more important article of diet than cow's milk, and as no kind of food is more subject to adulteration, it becomes a matter of greatest importance to public analysts as well as to milk consumers, that a safe and proper standard of the quality of cow's milk should be generally accepted.

Addition of water to milk is so common that there is scarcely any one who does not appreciate the old joke about " milk made " In the year 1883, of all the samples of milk analysed in London, (Analyst 8:243) 67.9% were below the limit because of water added,

20% were just on the limit line of solids (not fat) and fat in genuine milk, 19% were both skimmed and watered. For detection of water, it was important that the total solids including lactose should be accurately determined. Better methods have since been devised, but the determination of lactose still remains an important part of every milk analysis, especially in nutrition tests.

The average sugar content of milk is given by D'Hout as follows: for whole milk 4.70%, separated milk 5.05%, cream 3.32% The sugar content of milk depends in the highest degree upon the breed and care of the herd, especially upon the duration of the lactation period and upon the composition of the food. In the present paper we shall confine our attention entirely to the natural sugar content of cow's milk and its quantitative determination.

The estimation of lactose has been based for the most part, upon its power to rotate the plane of polarized light and its property of reducing many metallic compounds as copper, silver, and mercury in alkaline solution. In 1895, Blyth (Analyst 20: 121) announced that he considered the best method for determining lactose, to be a physical one supplemented by a copper process and when necessary obtaining the osazone. Such a physical method is the optical method; the copper process has two forms of application viz.- the gravimetric and the volumetric. A brief outline of the more important of these methods will be given.

OPTICAL.

The earlier optical work consisted chiefly in the study of the various modifications of milk sugar. In 1855 Erdmann showed that

ordinary milk sugar of its water of crystallization at 130° C. The rotation of the two forms are shown and the transition of the latter sugar showing bi-rotation over into the constant is clearly indicated.

Water - free Crystallised milk-sugar.		Ordinary milk - sugar deprived of water at 130°	
Immediate	39.5°	Immediate	84°
1 hr.	45.8°	1 hr.	73.4°
2 hrs.	49.6°	2 hrs.	67.3°
3 hrs.	52.2°	3 hrs.	62.9°
4 hrs.	53.9°	4 hrs.	60.1°
8 hrs.	56°	8 hrs.	56°

Erdmann designates three different modifications of anhydrous milk sugar, two of them solid and crystalline and the third existing only in solution.

a) First class obtained by quickly boiling ordinary solutions of milk sugar. Low rotatory power, which gradually rises. Very soluble in water.

b) Solid modification obtained by dehydrating ordinary milk sugar at 130° C Much less soluble than (a) and has high rotatory power which gradually diminishes until it is identical with the form (a)

c) An unstable liquid modification obtained by gradual transformation of the soluctions of (a) and (b) forms on keeping them. Stable only in solution for on crystallisation it changes in presence of water into ordinary milk sugar; while on driving off the water by boiling it gives the anhydrous form (a)

Schmoeger (Berichte 14: 2121) in 1880 gave a somewhat different classification of the modifications of milk sugar, involving five classes. He claimed credit for discovery of the phenomenon half-rotation. Schmoeger assigned the value for the constant specific rotation of lactose to be $(\alpha)_D$ 52.76°. Erdmann's work and also Schmoeger's was confirmed by Bechamp and also by Dubrunfaut.

Schmoeger further attempted to determine the relation between the rotatory power of milk sugar and the concentration of solution and the influence of temperature upon the power of rotation.
He used four different types of polarimeter, and observations led to the following conclusions.

1) The rotatory power of milk sugar up to a concentration $p = 36$ (parts by weight in 100 by weight of solution) is a constant.

2) As a mean for all observed values of $(\alpha)_D$ he gave
$(C_{12}H_{22}O_{11} + H_2O)$; $(\alpha)_D = 52.53°$ at 20° C

The greatest concentration he was able to obtain was 1 part sugar to 1.8 parts water ($p = 36$) Hesse also studied several dilute solutions. For his most concentrated solution $C = 12$g sugar in 100 c.c. solution, Hesse gave the value 52.4° at 20° C For very dilute solutions, he found higher values and it follows that the rotatory power of milk sugar decreases with increasing concentration. This work was verified by Tollens and Schmidt. Schmoeger found no material change for very dilute solutions.

For the specific rotatory power of lactose many different values have been given.

$(\alpha)_D$ = 54.2° by Poggiale
52.53° by Schmoeger
51.5° by Erdmann
52.67° by Hesse
53.8° by Hoppe-Seyler
52.47° by Berthelot
53.27° by Biot
52.53° by Parcus and Tollens
52.5° by Kjeldahl
52.5° by Wiley, etc.

Schmoeger's investigations show that the value $(\alpha)_D$ sinks with increasing temperature. The influence of changes in temperature under 20° is greater than at higher temperatures; and in the neighborhood of 20° C, $(\alpha)_D$ changes about 0.75° for 1° C variation. Denigés and Bonnans gave the formula for correction for a deviation of ρ near 20° : -

$$X = \rho - \frac{c}{1000} \times (20-t) = \rho \times \frac{1020-t}{1000}$$

Marked discrepancies in specific rotation of the same substance found by different observers do not always depend upon imputities in the material used, but very largely on improper manipulation of the polariscope or on variations in character of the sodium light used. The substance to be polarized must be colorless and clear as water. The angle of rotation is dependent on

1) Length of column read.
2) Wave length of light ray used.
3) On temperature of liquid.

As clarifying agents for milk there have been proposed: Sugar of lead and 10% alum solution by Hoppe-Seyler; lead acetate and acetic acid by Schmoeger, Pellet and Broquet; freshly prepared ferric hydroxide by Cornette; $CuSO_4$ by Soxhlet; meta-phosphoric acid by Denigés, Bigelow and McElroy; phosphotungstic acid by Schmoeger;

HgI_2 by Wiley; aluminium hydrate by Bigelow and McElroy; potassium mercuric iodide plus 20% H_2SO_4 by Scheibe; $Hg(NO_3)_2$ by Wiley and Ewell; acid mercuric nitrate by Patein and Dufan; trichloracetic acid by Obermayer and Wortmann; picric acid in acetic acid by Thibault and Gallien; phenol and sugar of lead by VanKetel; β-naphthol sulphuric acid by Riegler. Each reagent has been recommended by the originator as especially accurate.

In 1884, Wiley realizing that the methods then in vogue required too much time and labor, said, " If some reliable optical method could be devised, the determination of lactose would be the work of a few minutes. Little credit has hitherto been given to any of the processes of optical analysis in use." Determination of rotation is rendered difficult by presence of albumens which are left rotating. He showed that solutions of basic lead acetate are far from satisfactory. Acids and alkalies seem to increase the rotating power. As clarifiers Wiley used,

1) Saturated solution of basic lead acetate, sp.gr. = 1.97

2) Nitric acid solution of $Hg(NO_3)_2$ diluted with an equal volume of water.

3) Acetic acid, specific gravity 1.04 containing 29% acetic acid.

4) Nitric acid sp.gr. 1.197 containing 30% HNO_3

5) H_2SO_4 sp.gr. 1.255 containing 31% H_2SO_4

6) Saturated solution NaCl

7) Saturated solution $MgSO_4$

8) Solution of HgI_2 in acetic acid, formula; KI, 33.2g; $HgCl_2$, 13.5g ; strong acetic acid 20c.c. ; water 64Cc.c.

9) Alcohol, ether, and many solutions of mineral salts, HCl and

other acids were also tried. (Analyst 12:176)
He showed that excess basic lead acetate caused a rapid decrease in rotatory power. By combustion with soda lime, the quantity of laevorotatory matter remaining in filtrate after treatment with basic lead acetate was much greater than in those treated with the mercuric reagents. (Amer. Chem. Jr. 6:289) "This," he says, " explains the high percent of milk sugar obtained by using the mercury salts and shows that use of lead acetate must be abandoned." Excess of the mercuric salts does no harm and they can be used without heating the samples.

The method adopted by Richmond (Analyst 35:516) is to prepare the solutions with mercuric nitrate as usual and to add 5% of phosphotungstic acid and 5% of 1:1 H_2SO_4. After filtration, the solution is polarized and the readings multiplied by 1.1 Schmoeger (Analyst 11:143) has proposed correcting the reading of the polarimeter by making an allowance for the volume of precipitate obtained. This correction and conversion to per cent by weight, he would accomplish in one calculation by raising the reading of the polarimeter in the proportion 103:108. Little credit can be given to this method beacuse of the objection which has been raised to the originator's use of lead acetate. Vieth (Analyst 13:63) adopted the mercuric nitrate solution rather than subacetate of lead because he found it "more simple and expeditious." He says that a further calculation for variation in amount of fat must be made. The amount of sugar to be reduced by formula $\frac{100 \times \text{sp.gr.}}{100 - \text{vol.fat}}$. This was based upon the assumptions that

1) The volume of proteins in 100c.c. = 3c.c.

2) Vol. of fat in 100c.c. = % fat $\div$ .93

Richmond and Boseley (Analyst 22:98) used Vieth's method, but pointed out an error in the second assumption. They substituted for the volume of fat this formula : $- \left(\frac{\text{sp.gr. of milk} \times \text{\% fat}}{.93} \right)$

Raumer and Späth (Zeit Angew.Chem 1896:72) suggest boiling before polarizing, to prevent birotation. Their data are far from convincing for they clarified with lead subacetate. They make no attempt to prove the presence of the alleged disturbing dextrin which they agree with Ritthausen may be present. Scheibe sets forth (Analyst 26:127) a method for clarifying by means of H_2SO_4 and HgI_2 To 75c.c. milk add 7.5c.c. of 20% H_2SO_4 and 7.5c.c. of mercuric iodide solution, make up to 100c.c., filter and polarize. A correction for the error due to the volume of precipitate must be made. This is done by multiplying the result by .94 for whole milk and .97 for skim milk. An elaborate correction is worked out for cream, but will be omitted here.

Wiley and Ewell had previously allowed as correction for the volume of precipitate 2.6c.c. in 60c.c. of milk made up to 100c.c. volume. In 1896 they concluded that a constant factor could not be relied upon and proposed instead the " Double dilution and polarization " method originated by Scheibler.(Analyst 21:182) As a clarifier they used Wiley's $Hg(NO_3)_2$ solution diluted with five volumes of water instead of two as previously directed, because they found the more concentrated reagent did not give a prefectly colorless filtrate. The more dilute reagent throws out all proteids dissolved in milk and produces no inversion effect. It has been generally adopted by

chemists.

For double dilution and polarization, in each case double the quantity of milk for normal weight of the instrument used was taken. In one flask after clarification the solution was made up to 100c.c. and in the other to 200c.c. volume. Scheibler's method for calculation by multiplying the reading obtained from the solution in the large flask by two and subtracting from that obtained in the small flask was found to be inaccurate. They obtained the true polarization by dividing the product of the two readings by their difference. This method was tested out upon solutions containing definite amounts of insoluble materials such as quartz and sand and upon purchased milk and cream. Satisfactory results were obtained in all cases. Richmond (Analyst 22:98) defends Vieth's method. He states that the difference in results by Wiley-Ewell's method and by Vieth's method is due to large experimental error of the former method. Richmond considers double dilution method to be practically worthless on account of the large experimental error involved by its use. Waste of time and large amount of sample needed are further objections. Wiley and Ewell, however, consider their method to be "easy, rapid, accurate and equal in all respects to any gravimetric method whatever." They conclude their article with these words, "There seems to be no just reason for insisting on the slow and tedious gravimetric method when a quick and accurate optical method is at hand."

The optical method is considered uncertain by many investigators ; first, because the results often vary materially from those of the copper method, generally traced to presence of foreign optically active substances; second, because it is difficult to

precipitate the disturbing calcium salts and especially the albuminous matter completely and it is difficult to obtain a good, clear, polarisable filtrate. Scheibe (Zeit Ann. Ch. 1901 vol 40:1-14) suggests the removal of calcium salts in the gravimetric process in order to make the results check with those obtained by the polarimeter His method will be outlined under gravimetric methods.

Béchamp (Zeit Ann. Ch. 1901; 40:1-14) has come to the conclusion that polarimetric determinations of milk sugar in milk is just as faulty as determination by reduction. Raumer and Späth declare the polarimetric method to be inadmissable because of the right rotating bodies present. Knowles and Wilson (Jr. Chem. Soc. 1891:1298) compared the gravimetric determination with Fehling solution with the Pavy-Fehling method and with the optical method. They regarded the Pavy-Fehling method as rapid and accurate, but preferred the optical. There are objections to their results, because they clarified with acetic acid and lead subacetate.

GRAVIMETRIC.

Muter (Analyst 9:116) gives an account of the older methods for milk analysis in use before the passing of the Adulteration Act in 1872

I. A funnel with four high narrow ribs was fitted with filter paper and filled two-thirds of its height with sand (purified by washing in dilute HCl and igniting). This was placed in an air drying oven at 200°F. for some time, cooled 10 minutes in a dessicator and weighed. Enough milk was then dropped upon it so as to nearly saturate the sand without wetting the filter paper and the whole again weighed, then dried in the oven to constant weight always using the

same dessicator for same length of time at each weighing. Funnel and contents were then thoroughly percolated with boiling ether by placing in a tin jacket into which warm water was placed and then pouring on ether. It was finally removed from the jacket and again dried in the oven and the loss was considered to be the amount of fat. The residue was treated with water faintly acid or with very weak spirit to remove the soluble portion which was looked upon as crude milk sugar and then again dried. The balance was casein. Ash was taken on separate samples.

II: Another of the older methods was the evaporation of a portion of milk and dry residue at 220°F. and determination of ash in this residue. A separate sample of the milk was evaporated with plaster of Paris, being well stirred during the evaporation. The dry residue was reduced to powder in a glass mortar and extracted absolutely with boiling ether. The ether was received through a filter into a weighed, flat-bottomed flask, and after distilling off the ether the residual fat was dried at 220°F. and weighed. The plaster remaining in the basin and filter were now treated with water and the balance between the fat and sugar and total was casein.

Of all the methods studied for gravimetric determination of lactose those based upon its power of reducing copper salts in alkaline solution is the most important. For this purpose Fehling's solution is commonly used. In 1880, Muter concluded (Analyst 5:35) that

a) The best process for general use is the gravimetric.

b) It should be carried out according to strict rules so as to ensure a constant equivalent.

c) The precipitate may be directly weighed as Cu_2O. In his opinion, the volumetric method, in which the sugar must be brought into contact a little at a time with strong alkali, never gives really accurate results except by accident, and a really constant equivalent cannot be ensured unless the whole can be diluted to such a point as will render the alkali harmless, whilst the Fehling's solution is at once added in sufficient quantity to instantaneously perform the whole reaction, and both the sugar solution and the Fehling are actually boiled when mixed, points only to be attained by the gravimetric process.

The action of Fehling's solution with milk sugar has been investigated by many chemists; among them are Boedeker, Schiff, Rigaud, Städeler and Krause, Pellet and Biard, Pagnoul and Soxhlet. Very different results have been obtained in many cases. Much disagreement prevails in the number assigned as the copper oxide equivalent of milk sugar. Rigaud, Städeler and Krause, also Schiff found a proportion of 1:7, Boedeker at one time gave the result 1:7.5 and later 1:6&6/7 Soxhlet found value 1:7.39. Muter held proportion 1:7 . All are agreed that departing even in the least from exact details of procedure causes incorrect results.

[B. Herstein(Jr.American Chem.Soc. vol.32,no.6, page 779)]

To Trommer belongs the credit of having first introduced the alkaline copper sulphate solution into analytical chemistry, though it was at first used only for qualitative purposes. Trommer published his paper in 1841, on "The Differentiation between Dextrin, Cane, and Grape Sugar." He showed that in making the copper sulphate solution alkaline, it became a valuable reagent for the

differentiation between the various sugars. The necessity for an improved method of estimating sugars became so imperious that in 1838 a prize of 3000francs was offered, by the "Société d'encouragement pour l'industrie nationale", to the chemist who would devise a successful method for the quantitative estimation of sugar. In 1844, Ch. Barreswil submitted his solution. It was based upon Trommer's suggestion, and the improvement consisted in the addition of a solution of potassium tartrate to prevent the decomposition of the solution on heating. As proposed by Barreswil, the method was a quantitative volumetric one, the copper solution being standardized against a pure grape sugar solution of known strength, which latter was added drop by drop to the boiling copper solution, the end point of the reaction being reached when the blue color of the solution had disappeared.

H. Fehling, by whose name the method is apparently destined to remain known in chemistry, published his first paper concerning the subject, in 1848, in a medical publication, and only in 1849 did he give a more detailed account of his work to the chemical fraternity. Fehling's merit consisted chiefly in having worked out with great care, the details of the method, giving some account of the stoichiometrical equivalents, but his solution did not keep for any length of time and he did not recognize the fact that the reaction is quantitative only within very narrow restrictions as to the concentration of the solutions and the time of reaction. This point has been established only by the concerted efforts of the many chemists, who worked on the subject after Fehling, the necessity for keeping the copper solution divided into two parts being soon

recognized. Soxhlet established the exact conditions under which the determination must be carried out in order to get satisfactory results.

In determining lactose, by Fehling's solution, it is necessary to remove the proteins. Several methods have been suggested for their precipitation; acetic acid by Blyth, $CuSO_4$ by Soxhlet and boiling with caustic soda by Gescheidlen (Jr. Chem. Soc. 1878: 345)
In 1898 Riegler (Zeit Annal Chemie 37: 22) recommended asaprol reagent, prepared by mixing 15g. asaprol and 15g. crystalline(powdered) citric acid with 500c.c. distilled water and the resulting solution filtered. This reagent will keep indefinitely. 15c.c. of the reagent are placed in a 100c.c. volumetric flask with 10c.c. and solution made up to volume, shake vigorously, warm to about 60° and filter.

Pavy's solution may be used without removing the proteins. His solution is made up as follows : -

34.65g. crystallized $CuSO_4$
170g. Rochelle salts
170g. Caustic potash. Made up to a liter.

120c.c. of this solution with 400c.c. ammonia (to keep CuO in solution) are made up to a liter. This method is used most commonly to determine a mixture of sucrose and lactose and is a volumetric one. The titration is first made upon a sample of the milk as it is, then one portion is inverted by heating with 2% citric acid. The increase in reagent required after inversion is taken as a measure for cane-sugar as citric acid does not affect lactose in this reaction. The end point is difficult to obtain and the same objections are made to

this method as to all the volumetric ones.

The gravimetric method adopted by the A. O. A. C. is Soxhlet's method by means of Fehling's solution. The milk solution is prepared by diluting 25c.c. of milk with 400c.c. water adding 10c.c. $CuSO_4$ solution of strength given for Soxhlet's modification of Fehling's solution, adding 7.5c.c. KOH solution of such strength that one volume of it is just sufficient to completely precipitate the copper as hydroxide from one volume of the $CuSO_4$ solution. Instead of KOH solution of this strength, 8.8c.c. of a N/2 solution of NaOH may be used. After addition of the alkali, the mixture must still have an acid reaction and contain copper in solution. Fill the flask to the 500c.c. mark, mix, filter through a dry filter.

Denigés (Von Lippmann; Chemie der Zuckerarten) mixes 10c.c. milk with 25c.c. of 5% sodium-meta-phosphate solution and 60 - 70 c.c. water, adds 0.8c.c. acetic acid or 0.5c.c. HCl, made up to 100c.c. and heated with Fehling-Soxhlet's solution. Borntraeger prefers lead acetate as a clarifier, but supplements it with sodium sulphate or sodium phosphate. Peska worked out a table for determination of lactose by means of ammoniacal copper solution. In 1% solution 0.5g. milk sugar reduces 74c.c. Fehling's solution. For reduction of Knapp's or Sachsse's mercury reagents 311 and 465 mg.(respectively) of milk sugar in 1/2% solution are required, or in 1% solution 1g. milk sugar reduces 322.5c.c. of Knapp's and 214.5c.c. of Sachsse's mercurial reagents. The Sachss and Knapp reagents are reduced by creatin, glycerol, and even alcohol, so they are not very satisfactory

Soxhlet's modification of Fehling's solution is made up as

follows :

A. 34.64g. of pure crystallized $CuSO_4$ (free from iron and moisture) are dissolved in distilled water and the solution diluted to 500c.c.

B. 70g. NaOH not less than 97% NaOH.

(A. O. A. C. uses 50g. NaOH)

175g. recrystallised potassium sodium tartrate. Made up to 500c.c.

Mix equal volumes A and B immediately before use.

50c.c. of the mixed copper reagent are placed in a beaker and heated to boiling. While boiling briskly 100c.c. lactose solution are added containing not more than 0.8g. of lactose and boiled for 6 min. Filter at once through specially prepared asbestos and determine the amount of copper by one of the six methods.

1) Reduction of the Cu_2O in hydrogen.

2) Electrolytic deposition from H_2SO_4 solution.

3) Electrolytic deposition from H_2SO_4 and HNO_3 solutions.

4) Electrolytic deposition from HNO_3 solution.

5) Volumetric Permanganate method.

6) Direct weighing of Cu_2O.

Collect the precipitated Cu_2O on prepared asbestos felt, thoroughly wash with hot water, then with 10c.c. alcohol and finally with 10c.c. ether. Dry the precipitate 30 min. in a water oven, cool and weigh. The weight of Cu_2O multiplied by .8883 gives the weight of metallic copper. The weight of lactose equivalent to the copper reduced is found from the Soxhlet-Wein table.

Soxhlet's original article (Jr. Prak. Chem. 1880; 21:227-) makes reference to the method of Ritthausen for preparing the lactose solution from milk. Ritthausen, in 1877, originated the method of precipitating the protein from milk by means of $CuSO_4$ and alkali. He mentions use of tannin solution which completely precipitates proteins but gives a solution unsuitable for determination of milk sugar, because of the reduction of Fehling's solution by tannin Ritthausen studied the action of proteids as early as 1872 and concluded that : Legumin, conglutin, gluten-casein, milk casein and gliadin are precipitated from solutions unchanged and completely by copper oxide. By a series of investigations he showed that the protein bodies of milk, casein, protein and lactoprotein are completely precipitated by means of copper salts and potash, without change in their chemical constitution.

Ritthausen recommended use of 63.5g. $CuSO_4$ dissolved and made to 1 liter, 10c.c. of this corresponding to 0.2g. CuO . After addition of the copper reagent to the milk, such a quantity of potassium or sodium hydroxide should be added sufficient to decompose the copper salt used. He found it necessary to heat in the formation of the precipitates, and also suggested that if the milk contain triphosphate and free alkali then a part of the copper salt is thereby decomposed and as a rule only 7/10 of the calculated alkali is necessary to furnish the same quantity of CuO in the precipitation. " The solution should be neutral or slightly acid, If there is least excess of alkali the casein copper precipitate dissolves and the solution remains cloudy and contains a blue color.". If it is markedly acid, copper remains in solution, although separation of the precipitate

follows quickly and leaves a clear solution. To 20c.c. milk are added 10c.c. $CuSO_4$, the milk always diluted to 20 times its volume. After filtration, there will be contained only a minimum amount of copper in solution and the results will be accurate as checked by other methods of determination. The residual portion or precipitate is washed with water and the filtered solution in addition to the wash water serves for determination of milk sugar by Fehling's method. Protein and fat can be determined in the precipitate with complete accuracy.

Rodewald and Tollens (Berichte, 11:2076) first published their results concerning the reducing properties of milk sugar on alkaline copper solutions in 1878. They considered a revision of the older works upon milk sugar to be advisable, because considering the reducing power of dextrose as unity almost every chemist who had examined lactose had given different results for the amount of copper oxide corresponding to 1 molecule milk sugar; results varying from 6&6/7 to 8 moles. Chemists who had made many milk sugar determinations had given out different numbers at different times. They prepared their alkaline copper solution thus : -

A. 60g. best NaOH in sticks.
173g. crystallised Rochelle salts.
B. 34.639g. pure $CuSO_4$

Each solution made up to 500c.c. with distilled water. Of these equal parts were mixed immediately before use, and the required amount drawn out. None of the solutions were kept over 14 days after preparation. Other solutions of the same formula except 70g. NaOH instead of 60g. as above, were also tried. R. & T. are agreed with

Marcker and Holdefleiss and also with Soxhlet that the two solutions shall consist of one containing $CuSO_4$ and the other alkali and Rochelle salts; the two to be mixed instantaneously at the time of determination.

R. & T. tried Scheibler's gravimetric method in which the sugar is heated with excess of Fehling solution, the precipitated cuprous oxide converted into copper oxide. The method of Märcker and Holdefleiss in which the cuprous oxide was wet with nitric acid, evaporated and heated to glowing was found by R. & T. to give unsatisfactory results, and dissolving the entire precipitate of Cu_2O in nitric acid evaporating and heating to glowing was considered most troublesom and accompanied by unavoidable loss. Conversion of the cuprous oxide to Cu by means of hydrogen was only slightly less productive of loss due to small explosions and the escaping hydrogen sometimes burned with greenish flame. They adopted the method of taking the Cu_2O from the filter and placing the ignited filter ash in a tared porcelain boat. The boat was then placed in a wide glass tube with underlying platinum foil and the precipitate was reduced in a stream of purified hydrogen. After heating to complete reduction, the gain in weight of the boat indicated the amount of copper reduced. The slight precipitation of copper oxide by the paper found in the experiments of Märcker, Holdefleiss and Soxhlet was counteracted. Although this method gave satisfactory results, they still relied upon Soxhlet's method with the asbestos filtration as more convenient and rapid and since it eliminates the precipitation of copper by filter paper and oxidation and redissolving of Cu_2O is less likely to occur in a small asbestos

filter than on paper filter which offers a great amount of surface.

R. & T. found that three conditions are essential to accurate results by this method.

a) duration of heating. They recommend 4 min. heating on sand bath in preference to 10, 25, or 30 min. on water bath.

b) Concentration of the solution.

Other conditions being constant, one molecule milk sugar reduces different quantities of Cu_2O always according to concentration. Fehling solution diluted with

1 volume of water	7.34	atoms copper.
2 " "	7.46	" "
3 " "	7.47	" "
4. " "	7.53	" "
5 " "	7.43	" "
6 " "	7.44	" "

With only slightly diluted Fehling solution the milk sugar shows lower reducing power than with a greater dilution. It is probable that the stronger action of alkali upon the sugar which takes place in the concentrated solution destroys a certain amount of sugar before it has time to reduce copper oxide. By still greater dilution (6-9 parts of H_2O) of Fehling solution the precipitated quantity of Cu_2O falls off as if lessened reduction caused by deficiency in alkali or by dissolving again some cuprous oxide during the long duration of filtering of the great quantity of solution.

c) The excess of Fehling solution present.

One mole milk sugar reduced varying amounts of copper

according to excess of Fehling solution present. An increase from 160c.c. to 180c.c. Fehling's solution for 1g. milk sugar showed an increase of 1/10 of an atom of copper; thereby the filtrate remaining blue. When less copper solution was taken so that the filtrate was greenish blue or greenish yellow or contained so little copper that it only appeared brownish with H_2S, then less copper was reduced by 1 mole milk sugar.

Fehling solution diluted with the same volume of water showed the following results.

No. c.c. Fehling.	Atoms copper reduced.
145.8	7.23
146.1	7.27
149.7	7.25
152.8	7.35

R. & T. concluded that if necessary precautions are observed the gravimetric estimation of lactose is a completely useful and very accurate method and much to be preferred to titration alone. The checking of a great dilution and presence of a constant excess of Fehling solution is always necessary. 60g. of NaOH are to be preferred to 70g. as the increase in alkali causes a variation. In case of increased concentration of alkali, the sugar is practically destroyed before it can act upon the copper solution.

Four minutes boiling, filtration, reduction, etc. and subsequent multiplication of the copper obtained by .0763 is necessary. 1 mole milk sugar is found to reduce 7.47 atoms Cu. This method gives correct results. (Rodewald and Tollens)

Soxhlet (Jr. Prak. Chem. 21:227-317) found that the reducing power of sugar upon alkaline copper solutions is only constant under exactly the same conditions. If the same amount of sugar act in one case on an amount of copper solution which it is just able to reduce and in another on an excess, the reducing equivalent will in the first case be found to be considerably less than in the second. Evidently - if a solution of sugar is added by small quantities at a time as is necessary in ordinary volumetric estimation, the amount of reduction effected by the first quantities added will be greater than that produced by the last. To avoid this error, Soxhlet uses the sugar and copper solutions in the exact proportions necessary for their mutual reaction, determining the volumes necessary by a series of approximating experiments.

Invariable proceedure and special precautions are necessary. Apparently unimportant differences as time occupied in the experiment, amount of free alkali, presence of excess of the metallic solution, concentration of the liquid and other conditions liable to differ in every experiment, are all factors more or less concerned in the results obtained. Rigidly accurate results thus become impossible in many cases likely to occur in practical analyses. If the necessary precautions are taken, the gravimetric method with Fehling solution is very satisfactory.

In the Defren-O'Sullivan method, a modified Fehling solution is used. It consists of two solutions A. and B.

A.) Dissolve 34.64g. $CuSO_4$ in water and add 0.5c.c. strong H_2SO_4 and dilute to 500c.c.

B.) Dissolve 178g. sodium potassium tartrate and 50g. NaOH in water and dilute to 500c.c.

15c.c. of each of the above solutions are mixed in an Erlenmeyer flask, diluted with 50c.c. water, the flask placed on water-bath 5 min. then 25c.c. of a 0.5% solution of lactose are added and the flask left on water-bath 15 min. After filtration, the copper may be determined in any manner preferred.

Stokes and Bodmer devised a method using a solution (Analyst 10:62) which contains $CuSO_4$, KOH, Rochelle salts and ammonia. The use of ammonia was found to be objectionable by Richmond and Boseley and also by Hehner. The latter studied the above solution by replacing the KOH and Rochelle salts by sodium acetate, sodium carbonate, or NH_4Cl and showed that results obtained with the solutions made up in these ways were unreliable.

Pavy devised a modified Fehling solution (Analyst 10:62) as already described. Hehner has pointed out that Pavy's solution gives a tremendious variation in results with every alteration in conditions. Johnstone showed by experiments in 1885 that ammonia decomposes sugar of milk and it is therefore wholly inadmissable (Analyst 11:28)

In 1890, H. Ost (Berichte 23:3003) advised the use of a copper potassium carbonate solution, which contained 23.5g. ($CuSO_4 + 5H_2O$) 250g. K_2CO_3 and 100g. $KHCO_3$, in a liter. This solution according to Ost, is preferable to Fehling's solution in that

1) it keeps indefinitely,

2) it attacks cane sugar much less than Fehling's solution

3) The determinations with Fehling solution vary in the hands of

different chemists and only agree when same conditions are maintained. The determinations with copper potassium carbonate are much more reliable and agree better than Fehling's for different chemists.

4) From the copper potassium carbonate solution, the monosaccharides precipitate 1&1/2 - 2 times as much copper as from Fehling's solution, which essentially increases the accuracy.

5) It shows important differences for the reducing power corresponding to the several classes of sugar, making it easy to distinguish between them.

This method is not generally considered to have any advantage over Fehling-Soxhlet method due to the fact that it is much less accurate for milk sugar than for the monosaccharides. It is fairly accurate however, if the boiling of the solutions is prolonged ten minutes, and by use of an indicator may be used very successfully for volumetric work.

Kühn (Jr. Chem. Soc. 1891; 125) made the important suggestion that more concordant results are obtained by Fehling's solution when only so much serum solution is used that the liquid is bluish after boiling. If the filtrate is greenish, the results will be 0.1 - .15% too low, and less serum solution should be used.

Kjeldahl's method (Sherman Organic Analysis P. 74) involves three reagents.

1) Copper solution: 34.65 $CuSO_4$ in 500c.c.

2) 65g. NaOH (determined by titration) in 500c.c.

3) Pure sodium potassium tartrate, pulverized and kept dry.

To 5.2g. of the latter salt and 15c.c. of each of solutions 1) and 2)

are added a known volume of sugar solution which must not contain over 0.14g. dextrose or its equivalent. The mixture is placed in a 150c.c. Erlenmeyer flask, water is added to make 100c.c. solution, flask closed with two-holed stopper and hydrogen is passed in, to expell all air from the flask. Place the flask in the boiling water-bath 20 minutes, filter, and determine copper reduced.

The results obtained by the optical method are usually somewhat higher than are given by use of Fehling's solution. Many attempts have been made to correct the optical methods in various ways to bring a closer concordance with the gravimetric. Scheibe has suggested the correction of gravimetric methods by removal of calcium salts from the milk and he obtained concordant results in this way, i. e. by the optical and gravimetric methods. He demonstrated in 1901 (Zeit Ann. Ch. 40:1-14) that presence of calcium salts causes gravimetric results to be too low. He advised their removal in the following manner (Analyst 26:127) To 25c.c. of milk diluted with 400c.c water and treated with 10c.c. $CuSO_4$ portion of Fehling's solution are added 3.5-4 c.c. of normal NaOH solution and 20c.c. of a cold saturated solution of sodium fluoride; the liquid is allowed to stand 30 min. and then made up to 500c.c. and filtered. 100c.c. of filtrate are boiled 6 minutes with 50c.c. Fehling's solution and lactose corresponding to the amount of copper reduced obtained from Soxhlet's table.

Benedict (Jr. Biol. Chem. 3:103) has proposed the use of Na_2CO_3 in preparation of the alkaline copper solution instead of the strong caustic alkali used in Fehling's solution. The ordinary Fehling's solution does not keep well after mixing the two component solutions.

Benedict attributes this to the presence of Rochelle salt. As a substitute for the Rochelle salt, theoretically, any aliphatic compound which carries two or more hydroxyl groups may be used, provided it does not in itself reduce the copper at boiling temperature. Citric acid according to Benedict, is a most satisfactory reagent to use where a carbonate furnishes the alkalinity. His formula follows;

$CuSO_4$ 17.3g.

Sodium citrate 173g.

Na_2CO_3 100g.

Made up with distilled water to one liter.

Benedict's method is sometimes used for volumetric work but the end point is as hard to distinguish in his method as in any other.

EXPERIMENTAL.

As it has generally been agreed that either the gravimetric copper process or the optical method was the most satisfactory way in which to determine lactose, no volumetric work was done. The objection that optical and gravimetric methods do not give concordant results is quite common, so in beginning our work we had in mind the object of making an attempt to improve the gravimetric method in some way whereby results by the two methods might be obtained which would agree more closely than is commonly the case.

We first purposed to run a series of tests by the gravimetric and the optical methods with the object of finding out how closely the results by the two methods would check. The directions for proceedure were obtained from Bulletin 107 (revised) Bureau of

Chemistry, U. S. Dept. of Agriculture. On page 119 of that Bulletin is outlined the method for preparation of the milk solution for gravimetric estimation of lactose. The directions read as follows;

"Dilute 25c.c. of the milk with 400c.c. of water and add 10c.c. of a solution of $CuSO_4$ of the strength given for Soxhlet's modification of Fehling's solution, i.e. 34.639g. $CuSO_4.5H_2O$ made up to 500c.c. Add about 7.5c.c. of a solution of KOH of such strength that one volume of it is just sufficient to completely precipitate the copper as hydroxide from one volume of the solution of $CuSO_4$. Instead of a solution of KOH of this strength 8.8c.c. of a half-normal solution of NaOH may be used. After the addition of the alkali solution, the mixture must still have an acid reaction and contain copper in solution." These directions as outlined in the Bulletin are taken from Soxhlet's work (Jr. Prak. Chemie 21: 227) Soxhlet obtained his method from Ritthausen (Jr. Prak. Chem. 15: 329) This process is based upon the theory that 1) it is necessary to have alkali present to produce copper hydroxide from the $CuSO_4$, and that 2) the copper hydroxide thus formed is then the real precipitating agent for the proteins and the fats are carried down with them.

In following out these directions, we noticed that if the amount of alkali designated by the directions were added, the precipitate formed at first was re-dissolved and the blue color characteristic of the Biuret action was obtained. Leffmann (Analysis of Milk and Milk Products), is the only writer, whom we have been able to find who makes any reference whatsoever of this difficulty. He says, "The mixture should still have an acid reaction and contain copper in solution. If this is not the case, the experiment must be repeated,

using a little less of the alkali. Another trial must be made using less alkali. Now this method of trial and error is
1) a great waste of time and labor in actual practice and
2) requires a plentiful supply of the milk to be tested.

The classes in Chemistry 13a of some 125 students, at the University of Illinois were given samples of milk purchased in the open market and told to follow the directions in Bulletin 107. In every case, the addition of the quantity of alkali designated, caused a redissolving of the precipitate which formed readily at first on addition of the $CuSO_4$ as directed. Many trials had to be made before the correct amount of alkali to be added could be ascertained. When less alkali was added, there was still more or less trouble in filtration, sometimes two days were required to filter a sample made up to 500c.c. using a No. 595 S.& S. folded filter,

We noticed that by omitting the alkali entirely and making up the samples in all other respects as directed, we readily obtained a good flocculent precipitate, and a clear supernatant solution which was somewhat greenish in color and contained copper in solution, as specified by the Bulletin. This solution filtered very rapidly A great many different solutions were made up, always with the same results. So far as convenience is concerned there is no doubt but that omission of alkali is by far more satisfactory.

It then remained to be shown whether or not such a method would give accurate results as compared with the use of alkali, using the polarimeter as a means for checking up results. Several series of determinations were made preparing milk solutions with alkali and without. In all cases, the acidity of the milk was determined before

making up the solutions. The milk samples were measured with the same pipette which was accurately calibrated, and the weight of the samples calculated from the specific gravity of the milk. The two specifications in the Soxhlet method as given in Bulletin 107, are

1) the mixtures must still have an acid reaction and

2) must contain copper in solution.

To determine whether the solutions remained acid or not, the two indicators methyl orange and phenolphthalein were used to test portions of the filtrates obtained. In every sample where a good precipitation occured, whether alkali had been omitted or a minimum amount of alkali had been used, the solutions were neutral to methyl orange and acid to phenolphthalein. It seems conclusive then that if no more alkali is added than enough to neutralize the mineral acids as indicated by methyl orange, or if no alkali is added, provided the solution is neutral to methyl orange and acid to phenolphthalein, then under these conditions the precipitation will be complete, convenient and satisfactory. That there was copper remaining in solution could be detected by the color of the solution and the positive test with acetic acid and potassium ferrocyanide was shown.

It seemed possible that the omission of alkali might affect the accuracy of the subsequent determination of lactose in the solution thus prepared. From the data it can be seen that our results were just as satisfactory in all cases without use of alkali as with alkali. In general the results by the two methods of clarification check very well. Nitrogen was determined in the whole milk and in 100c.c. of filtrate in each case. The nitrogens check for the two methods pretty well generally. There were a few cases where an increase in

alkali showed a corresponding increase in nitrogen. This again favors omission of alkali.

The milk was purchased in the open market, carefully mixed and the specific gravity determined by means of a delicate hydrometer. A series of solutions were made up at the same time in some of which the alkali was omitted and in others varying amounts of alkali were used. We wish to emphasize the fact that in no instance were we able to add anything near the full amount of alkali prescribed by Soxhlet, without at once dissolving the precipitate and obtaining a cloudy solution with a blue Biuret color. As has been mentioned, solutions prepared with no alkali or with a minimum amount only filtered much more rapidly than those containing more alkali.

In order that the effect of the acidity of the milk might be noted, the acidity was always determined at the time the solutions were prepared. With considerable variation in acidity, no variation in the alkali that could be added was noticed. Milk having an acidity of 38°, the highest acidity of any experimented upon, was just as easily and accurately treated without alkali as any of those having low acidity. The standard for acidity was taken as the number of cubic centimeters of N/10 KOH necessary to neutralize 100c.c. of milk to phenolphthalein. Each c.c. was then represented by 1° acidity.

After addition of 10c.c. $CuSO_4$ solution to 25c.c. milk, diluted with 400c.c. water in a 500c.c. volumetric flask, alkali was added or omitted and volume made up to 500c.c., thoroughly mixed, allowed to settle and filtered through a dry folded filter. Lactose was determined in the filtrates by Soxhlet's method described previously under the heading gravimetric. The copper precipitates were

collected on prepared asbestos felts in Gooch crucibles, washed with hot water then with alcohol and ether, dried in a steam oven 1/2 hour cooled and weighed directly as Cu_2O In washing with ether, we noticed that care should be taken to add the ether only very slowly and with minimal amount of suction for ether evaporates rapidly enough to cause the felt to spring up out of the crucible, if these precautions are not observed, thus causing losses in Cu_2O

We then attempted to use a solution of tannin in alcohol as a clarifier, instead of Soxhlet's method discussed above. It was found to be satisfactory for complete precipitation of the proteins, but left a solution unfit for gravimetric determination of lactose by Fehling's solution since the reagent showed strong reducing power upon the Fehling at boiling temperature. We have since found that Ritthausen had already tried this in 1877, (J. Prak. Ch. 15:329) and that we had merely confirmed his results.

In preparing check solutions for optical determination of lactose the method of Wiley was used. The quantity of milk stipulated in the table was run from a burette into a flask calibrated to contain 102.6c.c. (Mohr), 15c.c. of Wiley's more dilute $Hg(NO_3)_2$ solution were added and volume completed to mark with distilled water. After filtration, the solution was polarized in a 200m.m. tube in a Schmidt and Haensch half-shadow saccharimeter, at a temperature of approximately 20° White light from a Welsbach gas burner was used and a light filter of $K_2Cr_2O_7$ solution was inserted. Perfectly clear and colorless solutions were obtained by means of the mercuric nitrate solution. While the more dilute solution i.e. diluted with 5 vols. water gave a clear colorless solution, it was demonstrated

that the stronger solution gave filtrates having a yellow tinge. The HgI_2 solution, formulated by Wiley, also gave a somewhat yellowish colored filtrate and was very much slower and less convenient than either of the $Hg(NO_3)_2$ solutions.

From the data which follows it may be seen that exactly concordant results could not be obtained by the optical and gravimetric processes. However, it will be noticed that the variation between results obtained by the two methods is practically uniform and that the variation is no greater where the milk solutions were prepared without alkali than where it was used. Thus it appears that results obtained without use of alkali are just as accurate as those obtained when alkali is used.

T A B L E I.

Sample	Wt.of 25c.c.	Acidity	No.c.c. Alkali added.	Wt.of Cu_2O	Wt.of Cu	%of Lactose.	Optical		Nitrogen Determinations.	
							Readings.	%of Lactose.	Description of Sample.	Wt. of Nitrogen.
I.	25.6824	24°	0	I.2840	.2522	3.62			5c.c. whole milk.	.0114
				II.2827	.2511	3.61	6.68	3.84	In 100c.c. filtrate I	.00616
II.	25.6832	24°	0	I.3032	.2693	3.88			II	.00495
				II.3010	.2673	3.85				
III.	25.6743	24°	1	I.2912	.2586	3.73			III	.00432
				II.2909	.2580	3.71				

As this was the first series in which nitrogen was determined, we do not believe it is entirely accurate.

Normality factor of NaOH added = .5553

T A B L E II.

							Optical		Nitrogen Determinations	
Sample Number	Acidity	Specific Gravity	No. c.c. of Alkali.	Wt. of Cu_2O	Wt. of Cu	%of Lactose.	Average Readings.	%of Lactose	Sample.	Wt. of Nitrogen.
IA	22°	1.0301	0	I.3649	.3241	4.72	10.1	5.05	5c.c. of whole milk	.0264
				II.3655	.3246	4.72			IA	.00512
IB		"	0	I.3636	.323	4.70			IB	.00506
				II.3629	.3223	4.68				
IIA	22°	"	0.5	I.3621	.3216	4.67			IIA	.0109
				II.3495	.3104	4.50				
IIB		"	0.5	I.3674	.3263	4.74			IIB	.0098
				II.3588	.3187	4.64				
IIIA	22°	"	1.5	I.3681	.3269	4.76			IIIA	.0119
				II.3682	.3269	4.76				
IIIB		"	1.5	I.3666	.3260	4.75			IIIB	.0055
				II.3727	.3310	4.82				
IV	22°	"	2	I.3671	.3260	4.74			IV	.0032
				II.3672	.3260	4.74				
V	22°	"	3.5	I.3679	.3268	4.74			V	.00414
				II.3656	.3250	4.73				

Normality of alkali added = .5553

TABLE III

Sample Number	Acidity	Specific Gravity	No. c.c. of Alkali	Wt. of Cu_2O	Wt. of Cu	% of Lactose.	Nitrogen Determinations. Sample.	Wt. of N_2
IA	17.4°	1.0285	0	I. .3638	.3231	4.71	5c.c. milk	.0214
				II. .3502	.3110	4.62	IA	.0069
IB			0	I. .3455	.3070	4.46	IB	.007
				II. .3416	.3040	4.42		
IIA		"	1	I. .3485	.3095	4.49	IIA	.0056
				II. .3511	.3118	4.52		
IIB			1	I. .3357	.298	4.33	IIB	.00655
				II. .3496	.310	4.51		
IIIA		"	2	I. .3438	.305	4.43	IIIA	.00655
				II. .3479	.309	4.49		
				III. .3451	.306	4.45		
IIIB			2	I. .3497	.3106	4.51	IIIB	.0069
				II. .3471	.3083	4.48		
IVA		"	3	I. .3554	.3157	4.60	IVA	.00655
				II. .3578	.3178	4.62		
IVB			3	I. .3530	.3135	4.56	IVB	.00655
				II. .3581	.3180	4.63		
VA		"	4	I. .3530	.3135	4.56	VA	.0138
				II. .3526	.3132	4.56		
VB			4	I. .3521	.3127	4.56	VB	.0149
				II. .3571	.3172	4.62		

T A B L E III continued.

Sample Number	Acidity	Specific Gravity	No. c.c. of Alkali	Wt. of Cu_2O	Wt. of Cu	% of Lactose	Nitrogen Determinations. Samples	Wt. of N_2
VIA	17.4°	1.0285	5	I.3459 II.3556	.3072 .3158	4.46 4.69	VIA	.0098
VIB			5	I.3462 II.3416	.3070 .3034	4.46 4.40	VIB	.0088

NOTE.- The solution prepared fot the determination of lactose by the optical method was lost.

The normality of the alkali = .5553

T A B L E IV.

Acidity	Specific Gravity	No. of Solution.	No. c.c. of Alkali	Wt. of Cu_2O	Wt. of Cu	% of Lactose	Optical		Nitrogen Determinations.	
							Readings	% of Lactose	Samples.	Wt of N.
16.55°	1.0275	IA	0	I. 3577	.3177	4.62			IA	.0056
				II. 3525	.3131	4.56				
		IB	0	I. 3464	.3077	4.47	8.7	4.65	IB	.00519
				II. 3425	.3086	4.48				
				III. 3469	.3081	4.48				
		II	2.35	I. 3483	.3093	4.50			II	.0056
				II. 3485	.3095	4.50				
		III	2.90	I. 3612	.3208	4.67			III	.0059
				II. 3530	.3135	4.58				
		IV	3.35	I. 3467	.3079	4.48			IV	.00632
				II. 3512	.3119	4.54				

Normality factor of Alkali = .949

T A B L E V

Series & Source.	No. of Sol.	Sp. Gr.	Acidity.	Grams Cu_2O	Grams Cu	% of Lactose	Optical Readings.	Optical % of Lactose	Nitrogen Determinations Samples	Nitrogen Determinations Grams of N.
A.	1.	1.0205	19.86°	I. .2492	.2213	3.18	6.630	3.315	5c.c. milk 1	.0047
				II. .2508	.2227	3.21				.0028
	2.	1.0235	19.86°	I. .3222	.2862	4.16	8.790	4.395	5c.c. milk 2	.0174
				II. .3208	.2849	4.14				.004
B.	1.	1.0235	38°	I. .2819	.2504	3.61	7.76	3.88	5c.c. milk 1.	.0202
				II. .2852	.2533	3.65				.0040
	2.	1.0275	38°	I. .3544	.3148	4.59	9.42	4.71	5c.c. milk 2	.0189
				II. .3561	.3163	4.60				.0035
C.	1.	1.0235	21.5°	I. .3049	.2708	3.93	8.190	4.095	5c.c. milk 1	.0230
				II. .3085	.2740	3.97				.0051
	2.	1.0270	21.5°	I. .3709	.3294	4.80	10.1	5.05	5c.c. milk 2	.0221
				II. .3707	.3292	4.80				.0037
D.	1.	1.0265	not determined	I. .3000	.2664	3.84	7.76	3.88	5c.c. milk 1	.0212
				II. .2969	.2637	3.81				.0018
	2.	1.0305	"	I. .3644	.3236	4.71	9.45	4.725	5c.c. milk 2	.0202
				II. .3590	.3196	4.65				.0021

No alkali was used in precipitation of any of the above.

NOTE - For explanation of table, see next page.

EXPLANATION of TABLE V

Sources of milk : -
A.Champaign Creamery
B.Kirkpatrick's
C.Fry's Clover Leaf Dairy
D.Old moldy milk.

Solutions No.I : - Made up by adding 50c.c. distilled water to 200c.c. milk.

Solutions No.II: - Made up by adding 50c.c. of a 5% solution of lactose to 200c.c. milk.

Lactose determinations made on 25c.c. samples made up to 500c.c. by Soxhlet's method.

Nitrogen determined in 5c.c. of milk and in 100c.c. of the prepared filtrates numbered 1 & 2

TABLE VI.

Acidity.		c.c. of Alkali.	Grams. of Nitrogen in 100c.c. Filtrates.		
			In 5c.c. whole milk.	=	.00972
23.12°	I	1.4c.c. HCl N.F. = .4114	I	=	.0058
	IIA	No alkali	IIA	=	.00279
	IIB	" "	IIB	=	.0031
	III	2.4c.c. NaOH sol N.F. = .4813	III	=	.00527
	IV	3c.c. above NaOH sol.	IV	=	.0071

DISCUSSION of TABLES.

In Tables I, II, III, IV, it will be noted that our results for per cent milk sugar obtained by the gravimetric method check very closely in most cases within 0.1% regardless of whethe the precipitation was made with ot without alkali. This seems to indicate that accurate results may be obtained equally well, when alkali is omitted in the clarification. That there is no great amount of protein matter remaining in the filtrates is indicated by the fact that the results in the nitrogen determinations gave results which were as concordant as could have been expected under the experimental conditions. In Tables III and IV, there is a slight increase of nitrogen shown in those filtrates prepared by using from 3-5c.c. of NaOH solution of N.F. = .5553

Table V shows the application of our method of precipitation to eight samples of milk from four different sources. Not only are the percentages of sugar obtained by gravimetric process concordant, but they check very satisfactorily with those obtained by the optical method.

Table VI shows a series of nitrogen determinations made to determine the effect of the quantity of alkali added upon the amount of nitrogen left in the filtrate. There seems to be an increase in the amount of nitrogen remaining in the filtrate when the acidity is materially increased by addition of HCl and also when alkali in the form of NaOH solution is added.

CONCLUSIONS.

1) We conclude therefore that precipitation of proteid matter from milk is much more convenient by means of $CuSO_4$ solution alone than by subsequent addition of alkali.

2) That use of small amounts of alkali does not affect the ease of precipitation, provided that the solutions remain neutral to methyl orange and acid to phenolphthalein.

3) Use of the amount of alkali stipulated by the A.O.A.C. is impossible.

4) Use of more than the minimum quantity of alkali tends to cause a dissolving of the copper compound first formed.

This same objection has been made to Stutzer's method of precipitation of proteins (Jr. Am. Chem.Soc. 22:709)

" This difficulty arose in many cases even when care had been taken to avoid the presence of free alkali "

5) We maintain that the copper albuminate is formed equally well with $CuSO_4$ alone as it is with $Cu(OH)_2$ and moreover is precipitated more readily as danger of dissolving the precipitate is avoided.

6) Results obtained on the filtrates prepared by our method are just as accurate in all cases as those prepared by use of alkali;

a) Good checks were obtained by the two methods.

b) We were able to get results by the optical method which checked within 0.2% with those obtained by the gravimetric method on samples prepared without use of alkali.

c) In a few cases there was more nitrogen left in the filtrates prepared by use of alkali than in those prepared without alkali.

It may be later demonstrated that our method is even more accurate than the old method in which precipitation was effected by addition of alkali.

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